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DOXORUBICIN RESISTANCE CONFERRED BY SELECTIVE ENHANCEMENT OF INTRACELLULAR GLUTATHIONE PEROXIDASE OR SUPEROXIDE DISMUTASE CONTENT IN HUMAN MCF-7 BREAST CANCER CELLS

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To examine the role of doxorubicin-stimulated oxyradical formation in tumor cell killing, we introduced glutathione peroxidase (GSH Px) or superoxide dismutase (SOD) into MCF-7 cells by "scrape loading." Control cytoplasmic GSH Px and SOD levels increased from (mean ± S.E.) 0.37 nmol/min/mg and 0.58 μg SOD/mg, respectively, to 3.99 or 7.63 nmol/min/mg and 1.40 or 1.83 μg SOD/mg after treatment with either 150 or 300 units/ml of GSH Px or 20 or 40 mg/ml SOD. Resistance to doxorubicin was correlated with the level of GSH Px introduced into the MCF-7 cells; a one-hour exposure to 1.75 μM doxorubicin decreased the cloning efficiency of control cells loaded with medium alone to 34%, whereas doxorubicin-treated cells augmented with 150 or 300 units/ml of GSH Px had plating efficiencies of 56 or 86%, $P < 0.05$. Introduction of SOD increased MCF-7 resistance to doxorubicin similarly. The heat-inactivated enzymes were not protective. Cells loaded with GSH Px were also resistant to the redox cycling anticancer quinone mitomycin C but not to the redox inactive analogs 5-iminodaunorubicin and mitoxantrone, suggesting that amplification of GSH Px or SOD levels can produce doxorubicin resistance in MCF-7 cells.

KEY WORDS: Drug resistance, glutathione peroxidase, superoxide dismutase, doxorubicin, mitomycin C, guinones.

INTRODUCTION

Recent studies have shown that resistance to the anticancer quinones *in vitro* may take the form of overexpression of antioxidant gene products.[1,2] In prior studies demonstrating changes in the peroxide handling capacity of human tumor cells, however, resistance to the anticancer quinones was obtained by stepwise selection with increasing concentrations of doxorubicin in cell culture, leading to the development of resistant cell lines displaying a wide variety of genotypic and phenotypic alterations including the presence of the P-glycoprotein.[3] In this study, we have examined whether a single biochemical alteration–specifically, enhancement of the level of oxyradical detoxifying enzymes GSH Px or SOD–could produce breast cancer cells that were resistant to the cytotoxic effects of anticancer quinones known to generate a reactive oxygen flux *in vivo*.

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MATERIALS AND METHODS

Human MCF-7 breast cancer cells were maintained in cell culture and used for cytotoxicity assays as previously described.[4] The permeability of the plasma membrane of the MCF-7 cells was transiently altered at 4°C by scraping tumor cells in logarithmic-phase growth from plastic tissue culture flasks with either a rubber policeman or a plastic scraper. Using the technique of McNeil and colleagues,[5] human erythrocyte glutathione peroxidase or bovine erythrocyte superoxide dismutase (both from Sigma Chemical Co., St. Louis, Missouri) were introduced into MCF−7 cells. Enzymes assays on tumor cell cytosol were performed as described in a prior publication.[6] Chemotherapeutic drugs were obtained either from commercial sources or from the DCT, NCI, NIH.

RESULTS

Cytoplasmic glutathione peroxidase levels could be increased up to 20-fold during the process of mechanical disruption of the tumor cell plasma membrane. GSH Px activity increased from 0.37 nmol/min/mg in control cells to 3.99 or 7.63 noml/min/mg after scrape loading with 150 or 300 units/ml of GSH Px in the tissue culture media. As shown in Table I, enhancement of intracellular GSH Px activity almost completely eliminated doxorubicin-related cytotoxicity at the 50% inhibitory concentration of this drug. Furthermore, there was a clear relationship between the increase in cytoplasmic enzyme level and the protective effect of GSH Px. We also found that MCF-7 cells loaded with GSH Px were resistant to mitomycin C and menadione. However, neither heat-inactivated enzyme nor GSH Px present in the media without cell membrane permeabilization had any effect on tumor cell killing by doxorubicin. The cytotoxicity of the redox inactive anthracycline analogs 5-iminodaunorubicin or mitoxantrone was not affected by the introduction of GSH Px into MCF-7 cells. Introduction of GSH Px into MCF-7 cells had no effect on the uptake or efflux of doxorubicin.

In related experiments, SOD was mechanically loaded into the human breast carcinoma cells. Cytoplasmic SOD increased from 0.58 μg SOD/mg in control cells to 1.40 or 1.83 μg SOD/mg in cells loaded with 20 or 40 mg/ml SOD. At the higher level of SOD, tumor cell killing by 1.25 μM toxorubicin was abolished. Heat inactivation of the SOD prior to scrape loading completely inhibited its effect on the cytotoxicity of doxorubicin.

TABLE I
Effect of glutathione peroxidase on doxorubicin cytotoxicity in MCF-7 cells

Reaction system	Cloning Efficiency (% Control ± S.E.)
Doxorubicin (1.25 μM)	54 ± 2
+ GSH Px (300 units/ml)	88 ± 4*
Doxorubicin (1.75 μM)	34 ± 5
+ GSH Px (150 units/ml)	56 ± 2*
+ GSH Px (300 units/ml)	86 ± 2*

*$P < 0.05$ compared to cells loaded with medium alone for a minimum of 3–5 experiments.

DISCUSSION

Recent studies from our laboratory[4] suggested that exposure of human MCF-7 cells to exogenous catalase or superoxide dismutase protected these cells from the cytotoxicity of doxorubicin. Hence, we were interested in furthering these investigations by altering the *intracellular* antioxidant milieu of this human carcinoma cell line. In the present experiments, we have demonstrated that selective augmentation of intracellular peroxide or superoxide detoxifying activities by approximately 5- to 10-fold leads to an approximate 3- to 5-fold increase in the IC_{50} concentration for doxorubicin in MCF-7 cells. These changes occurred in the absence of other phenotypic alterations in the tumor cells, and thus, suggest that drug-related redox cycling may play an important role in the antineoplastic activity of the anthracycline antibiotics and related anticancer quinones including mitomycin C and menadione. Furthermore, the change in IC_{50} concentration noted with the introduction of the highest level of GSH Px tested is within the range of concentrations that under clinical circumstances would render a patient resistant to the anthracycline antibiotics. Thus, modest changes in the processing of drug-related reactive oxygen metabolities could prove to be an important mechanism of resistance to this class of therapeutic agents *in vivo*.

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